Berne virus possesses haemagglutinating activity which is inhibited by antisera that neutralize the infectivity of the virus. In decreasing order, human, rabbit and guinea-pig erythrocytes were agglutinated whereas agglutination was not observed with rat, goose, chicken or horse red blood cells. This pattern is different from that seen with the closely related Breda virus of cattle. Haemagglutinin was found to co-sediment with viral infectivity in sucrose density gradients. Transmission electron microscopy showed that intact virus particles form bridges between adjacent erythrocytes. The viral envelope was seen at a distance from the erythrocyte surface suggesting that the peplomers possess haemagglutinating activity. Haemagglutination was decreased in the presence of fetuin and gangliosides and also by pretreatment of the erythrocytes with periodate, suggesting that the virus binds to glycoproteins and/or glycolipids on the erythrocyte surface.

Berne virus is a member of the newly proposed Toroviridae family which encompasses a group of antigenically related RNA viruses of horse, cattle and man (Horzinek & Weiss, 1984). Toroviruses possess a spike-bearing envelope and are either kidney-shaped particles of diameter 120 to 140 nm or of rodlike appearance (35 by up to 180 nm) (Weiss et al., 1983). Although Berne virus was originally isolated from a horse with diarrhoea, its pathogenic potential is unknown at present. Serological evidence suggests that infection with this or similar viruses may be widespread in horse, cattle, pig, sheep and goat.

Berne virus is serologically related to Breda virus (Weiss et al., 1983, 1984; Koopmans et al., 1986) which was isolated from diarrhoeic calves by Woode et al. (1982). The close relationship between Berne and Breda viruses is evident also from the similar electrophoretic mobility in SDS–PAGE of Berne and Breda virus proteins. Purified Berne virus possesses four protein species of Mr 20000 (20K), 22K, 37K and 75K to 100K (Horzinek et al., 1984, 1985) and in Breda virus proteins of 20K, 37K, 85K and 105K were detected (Koopmans et al., 1986). Breda virus has been shown to agglutinate rat erythrocytes (Woode et al., 1982), but haemagglutinin has not been detected in Berne virus. The failure of Berne virus to agglutinate red cells has previously been attributed to the relatively low particle numbers obtained in cell culture as compared to the very significant virus concentrations observed in the faeces of calves infected with Breda virus (Horzinek & Weiss, 1984).

We report here that Berne virus agglutinates human, rabbit and guinea-pig erythrocytes. Haemagglutination is inhibited by antisera to Berne virus. In addition, we present evidence suggesting that the human erythrocyte receptors for Berne virus are glycoproteins or glycolipids.

Berne virus-infected cells were earlier shown to be positive for haemadsorption with guinea-pig erythrocytes (F. Steck et al., unpublished). We therefore screened erythrocytes of various animal species for binding to Berne virus-infected cells. Equine dermis cells originally obtained from the American Type Culture Collection were grown in microtitre plates (Greiner, Nürtingen, F.R.G.) and semi-confluent monolayers were infected at a multiplicity of 1
Table 1. HI and neutralizing activities of antisera to Berne virus

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>HI titre*</th>
<th>Neutralization titre†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse-anti Berne virus (P175/83)</td>
<td>320</td>
<td>648</td>
</tr>
<tr>
<td>Negative horse serum (P228/83)</td>
<td>10</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Rabbit-anti Berne virus</td>
<td>320</td>
<td>1612</td>
</tr>
<tr>
<td>Monoclonal mouse antibody to Berne virus</td>
<td>5000</td>
<td>25000</td>
</tr>
<tr>
<td>virus (11A1D2H1, ascites)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Haemagglutination inhibition (HI) assays were carried out in U-bottom microtitre plates (Greiner, Nürtlingen, F.R.G.). Sera (25 μl) were diluted in twofold steps in PBS and then incubated with 4 HAU of Berne virus (25 μl). To detect haemagglutinin, 50 μl of 0-5% human O blood group erythrocytes in PBS containing 1% bovine serum albumin was added after incubation of serum with virus for 1 h at room temperature.

† Neutralization assay was as described by Weiss et al. (1984), using 10^2 TCID₅₀ of Berne virus.

TCID₅₀/cell. Forty h post-infection the medium was removed and 100 μl of a 0-05% (v/v) erythrocyte suspension in saline was incubated with the cells at 4 °C for 20 min. Subsequently the cell monolayers were washed twice with phosphate-buffered saline (PBS). Human (blood group O), rabbit and guinea-pig erythrocytes became bound to the infected cells. In contrast, rat, goose, chicken, mouse, sheep, cattle and horse red cells were negative in this assay. Haemagglutinin was also detected in supernatants derived from Berne virus-infected cell cultures. A suspension of Berne virus containing 10⁷ TCID₅₀/ml agglutinated human, rabbit and guinea-pig erythrocytes with titres of 8, 4 and 1, respectively.

The specificity of haemagglutination was demonstrated in haemagglutination inhibition (HI) assays using antisera to Berne virus. Horse and rabbit sera with high titres of neutralizing antibody inhibited haemagglutination, whereas low HI titres resulted with sera negative in the neutralization test. Moreover, a monoclonal antibody to Berne virus (11A1D2H1, kindly supplied by Dr B. Kaeffer), inhibited both the infectivity and haemagglutination of Berne virus (Table 1); this antibody has been shown to recognize the 75K to 100K glycoprotein in radioimmunoprecipitation (Horzinek et al., 1986).

A supernatant from a Berne virus-infected cell culture containing 8 haemagglutinating units (HAU)/25 μl was concentrated to 128 HAU/25 μl by differential centrifugation. This 16-fold concentrated preparation also failed to agglutinate erythrocytes of SIV and Wistar rats. This observation, together with the previous finding that Breda virus does not agglutinate human and guinea-pig erythrocytes (Woode et al., 1982), clearly shows that the two haemagglutinins differ in their erythrocyte receptor-binding sites.

The pellet obtained after ultracentrifugation contained in addition to concentrated haemagglutinin >99% of the infectivity of the original cell culture supernatant. This preparation was subjected to isopycnic centrifugation in a linear 15 to 50% (w/w) sucrose gradient in a SW41 rotor at 30000 r.p.m. for 5 h. Fig. 1 shows that the position of the haemagglutinin correlated with that of viral infectivity, being at a density of 1-15 to 1-18 g/ml. This result suggests that the haemagglutinin is part of the virus particle.

Additional evidence for this interpretation was obtained by electron microscopy of erythrocyte agglutinates. Fig. 2 shows Berne virus particles bridging two erythrocytes. The viral membrane is seen at a distance of 18 ± 3-4 nm (n = 37) from the erythrocyte membrane. Since the unfixed peplomers of Berne Virus were shown to measure 20 nm in length in negatively stained preparations (Weiss et al., 1983) this suggests that the haemagglutinating activity is due to the viral spikes formed by the 75K to 100K glycosylated protein (Horzinek et al., 1986).

To characterize the nature of the surface receptor(s) of Berne virus, we investigated the effects of various erythrocyte pretreatment schemes and studied haemagglutination in the presence of inhibitors known to interfere with the binding of influenza viruses to the red cell surface (for review, see Bächli et al., 1977). For comparison, influenza A/swine/Germany/2/81 (H1N1) was tested in parallel with Berne virus. As shown in Table 2 haemagglutination was reduced by pretreatment of the erythrocytes with 0-5 mm-periodate and in the presence of bovine brain
Fig. 1. Density gradient centrifugation of Berne virus. The virus was concentrated from tissue culture supernatant by differential centrifugations at 4000 g for 15 min, followed by centrifugation in a Beckman SW28 rotor at 27000 r.p.m. for 90 min. The pellet was resuspended and layered on top of a linear 15 to 50% (w/w) sucrose gradient in TES buffer (0.01 M-Tris-HCl, 0.001 M-EDTA, 0.15 M-NaCl, pH 7.4) and centrifuged for 5 h at 30000 r.p.m. in a SW41 rotor. Infectivity titration (■) was as previously described (Weiss et al., 1983). Agglutinating activity (□) was measured as described in the text.

Fig. 2. Berne virus particles bridging human erythrocytes. Bar marker represents 100 nm.

gangliosides or fetuin. With the exception of fetuin, inhibition was more pronounced with influenza virus than with Berne virus. Neuraminidase pretreatment of the erythrocytes resulted in a 16-fold reduction in agglutination by influenza virus but had no effect on haemagglutination by Berne virus. A wide range of proteolytic enzymes (trypsin, α-chymotrypsin, papain, proteinases A and K and Pronase E at 0.1 mg/ml) failed to affect haemagglutination by Berne and influenza viruses (results not shown). Taken together, these observations suggest that Berne virus binds to surface structure(s) containing carbohydrates, most likely to glycolipids.
Table 2. Effects on Berne virus haemagglutination of gangliosides and fetuin and of erythrocyte pretreatment by periodate and neuraminidase

<table>
<thead>
<tr>
<th>Erythrocyte pretreatment or inhibitor</th>
<th>Berne virus</th>
<th>Influenza A/swine/Germany/2/81</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Gangliosides†</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Fetuin†</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Periodate‡</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td>Neuraminidase‡</td>
<td>100</td>
<td>6</td>
</tr>
</tbody>
</table>

* Haemagglutination assays were carried out in U-bottom microtitre plates using PBS as the diluent. Virus was diluted in twofold steps in 25 µl to which 25 µl PBS and 50 µl 0.5% human erythrocytes in PBS containing 1% bovine serum albumin were then added. One haemagglutinating unit is defined as the dilution showing complete agglutination and for ease of comparison is indicated as 100%.

† Gangliosides (Sigma, type III) and fetuin (Sigma, type III) were present in the haemagglutination buffer at 0.5 mg/ml and 1.25 mg/ml, respectively.

‡ Erythrocytes were pretreated as described by Armstrong et al. (1984). Briefly, 0.5 ml of a 5% erythrocyte suspension was incubated with periodate (final concentration 0.5 mM) for 1 h or with neuraminidase (final concentration 7 units/ml) at 37 °C for 2 h. The cells were then washed twice in Eagle’s MEM and suspended in PBS.

However, glycoproteins might also serve as receptors for Berne virus because it cannot be ruled out that glycoproteins were removed incompletely by the proteases, and/or that glycolipids became accessible for binding after removal of glycoproteins. Moreover, due to the limited specificity of the Clostridium perfringens neuraminidase employed in our experiments, neuraminic acid cannot be excluded at present as a carbohydrate constituent of the Berne virus receptor(s).

We thank Dr B. Kaeffer, Utrecht, The Netherlands for a generous gift of monoclonal antibodies to Berne virus. We also thank M. Jakob for her expert technical assistance.

REFERENCES


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